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Structure-based design and synthesis of pyrrole derivatives as MEK inhibitors

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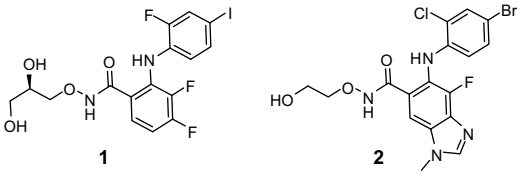
ABSTRACT

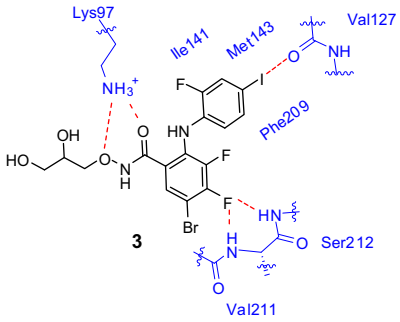
A novel series of pyrrole inhibitors of MEK kinase has been developed using structure-based drug design. Optimization of the series led to the identification of potent inhibitors with good pharmaceutical properties.

The mitogen activated protein kinase (MAPK) signaling pathways are involved in controlling cellular processes including proliferation, differentiation, and apoptosis.[1] The RAF-MEK-ERK signal transduction pathway plays a central role in mediating the transmission of growth-promoting signals from several growth factor receptors. MEK occupies a strategic downstream position in the RAF-MEK-ERK pathway, catalyzing the phosphorylation of its only known substrates, ERK1 and ERK2.[2] Overexpression and activation of MEK/ERK have been associated with several types of human cancers.[3] The inhibition of MEK has the potential to control cell growth and provide a treatment for various cancers.[4]

Several MEK inhibitors have been evaluated in the clinic, including PD0325901 (**1**) and AZD6244 (**2**).[5] The reported X-ray co-crystal structure of a close analogue of PD0325901 (**3**) demonstrates this class of inhibitors binds in an allosteric pocket adjacent to the ATP binding site,[6] showing key H-bonding interactions of the hydroxamate oxygens with Lys97, and H-bonding of a fluorine with the backbone NHs of Val211 and Ser212. The iodo group makes an important electrostatic interaction with the backbone carbonyl of Val127. Hydrophobic interactions in the pocket formed by Ile141, Met143, Val127, and Phe209 are also observed.

We set out to design novel MEK allosteric site inhibitors that would incorporate the key interactions with Lys97 and the hydrophobic pocket, while making the important Val211 and Ser212 H-bonding interactions through an exocyclic carbonyl on a five-member ring heterocyclic scaffold. We initially tested this idea with the 3-aminopyrrole compounds **8a** and **8b**, which were synthesized according to the general method depicted in Scheme 1. The Michael addition of an *N*-acetylamino acid methyl ester to *tert*-butyl acrylate in the presence of potassium *tert*-butoxide proceeded with a subsequent Dieckmann cyclization to form the β-keto ester compound **4**.[7] Acid catalyzed condensation with 2-fluoro-4-iodoaniline was followed by tautomerization to the enamine **5**,[8] which was oxidized with DDQ to produce the pyrrole compound **6**. Finally, acidic hydrolysis of the *tert*-butyl ester gave the carboxylic acid **7**, and hydroxamic ester formation by HATU coupling led to the target compounds **8a** and **8b**.





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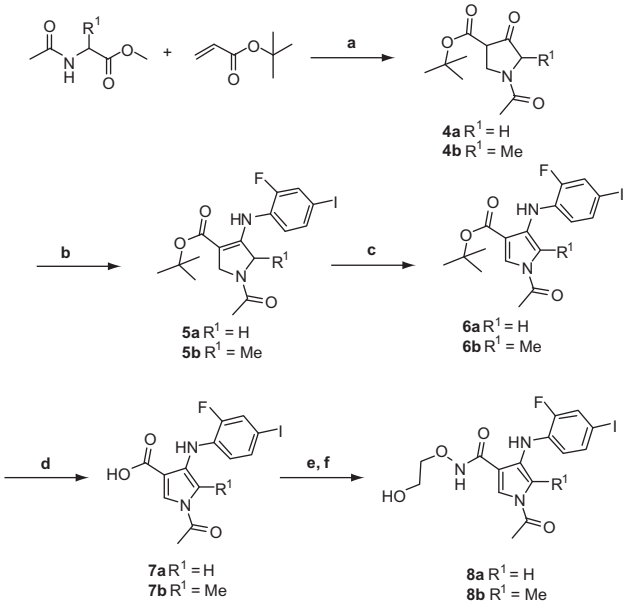
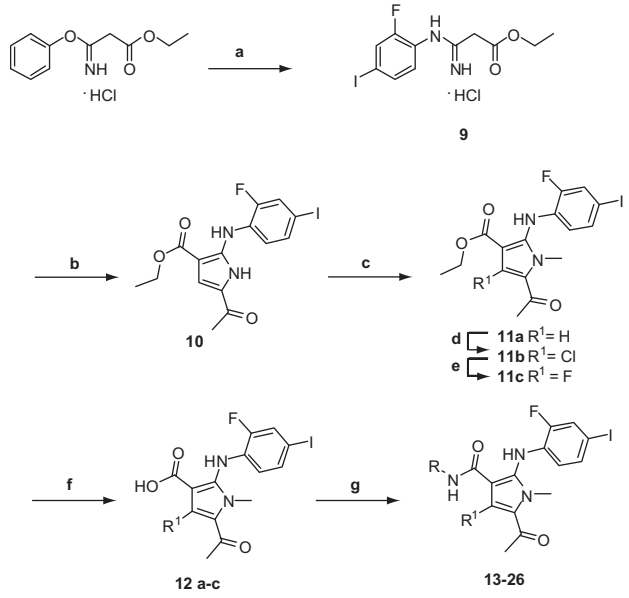


Scheme 1. Synthesis of compounds **8a** and **8b**. Reagents and conditions: (a) KO*t*Bu, THF, rt, 18 h; (b) 2-fluoro-4-iodoaniline, *p*TsOH, benzene, reflux, 1 h; (c) DDQ, toluene, reflux, 30 min; (d) 50% TFA/CH_2Cl_2, 4 h; (e) $CH_2CHOCH_2CH_2ONH_2$, HATU, CH_2Cl_2; (f) 25% 1 N HCl/THF, 1 h.



Scheme 2. Synthesis of compounds **13**–**26**. Reagents and conditions: (a) 2-fluoro-4-iodoaniline, EtOAc, reflux; (b) 1-bromo-2,3-butanedione, THF, reflux; (c) Me_2SO_4, Cs_2CO_3, DMF; (d) *N*-chlorosuccinimide, CH_2Cl_2; (e) CsF, DMSO, 180 °C; (f) NaOH; (g) R-NH_2, HATU, CH_2Cl_2.

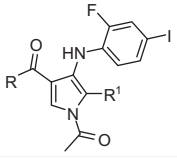
The *N*-acetyl pyrrole compounds **8a** and **8b** (Table 1) demonstrated excellent enzymatic activity,[9] but showed only weak, micromolar activity in our cellular assays.[10] This was due to the inherent chemical instability of the series: pyrrole N-deacetylation was observed over the course of the assay incubation. To address this issue, it was necessary to modify the central core while retaining all of the key binding elements. We envisioned this could be achieved with a modification to a 2-aminopyrrole scaffold (shown in compounds **13**–**26**).

The synthesis of 2-aminopyrrole compounds **13**–**26** is outlined in Scheme 2.[11] 2-Fluoro-4-iodoaniline was heated with ethyl 3-imino-3-phenoxypropionate hydrochloride to give the imidate salt **9**.[12] Reaction with 1-bromo-2,3-butanedione[13] led to the formation of the substituted pyrrole scaffold **10**. Pyrrole N-methylation with dimethylsulfate gave compound **11a**. Optionally, the 4-position of the pyrrole could be chlorinated with NCS to give **11b**, which could further be converted to the fluoride **11c** by microwave heating in the presence of cesium fluoride. Finally, ester hydrolysis to the carboxylic acid **12** followed by amide or hydroxamic ester formation produced the target compounds **13**–**26**.

The in vitro data for compounds **13**–**26** is presented in Table 2. Compound **13** showed comparable enzyme activity to its pyrrole isostere **8b**, but also displayed potent cellular activity. The activity for the diols **15** and **16** were comparable to the alcohol **13**, suggesting limited productive interaction of the additional hydroxy group with the ATP phosphates. However, the diols showed improved microsomal stability and solubility. Activity of the *S*-isomer diols was only marginally better than the *R*-isomers for this series. Addition of a halide group (F, Cl) at the pyrrole 4-position had little effect on activity. The amide **18** was less potent than its corresponding hydroxamic ester **13**, but amide R-groups were well tolerated when incorporated in a 4- or 5-member ring (**24**–**26**). Compounds from this series did not inhibit other kinases tested with inhibitor concentrations up to 10 μM.[14]

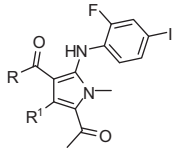
The pharmacokinetic properties of several compounds were determined in rats (Table 3). The alcohol **13** showed medium to

Table 1
Selected data for *N*-acetyl pyrrole analogues



Compd	R	R^1	MEK1 IC_{50}[9] (μM)	Colo205 EC_{50}[10] (μM)	HLM/RLM $t_{½}$ (min)
7a	OH	H	0.220	>50	29/20
7b	OH	Me	0.072	21	29/21
8a	$NHO(CH_2)_2OH$	H	0.011	3.1	3/6
8b	$NHO(CH_2)_2OH$	Me	0.020	10	8/2

Table 2
Selected data for 5-acetyl *N*-methyl pyrrole analogues



Compd	R	R^1	MEK1 IC_{50}[9] (μM)	Colo205 EC_{50}[10] (μM)	A375 EC_{50}[10] (μM)	HLM/ RLM $t_{½}$ (min)
12a	OH	H	0.042	7.10	2.65	123/7
13	$NHO(CH_2)_2OH$	H	0.018	0.012	0.014	36/14
14	$NHO(CH_2)_3OH$	H	0.046	0.104	0.176	43/13
15	(*R*)-$NHOCH_2CHOHCH_2OH$	H	0.020	0.033	0.036	>200/29
16	(*S*)-$NHOCH_2CHOHCH_2OH$	H	0.011	0.011	0.017	150/68
17	$NHOCH(CH_2OH)_2$	H	0.019	0.020	0.033	90/16
18	$NH(CH_2)_3OH$	H	0.090	0.128	0.313	95/10
19	$NHO(CH_2)_2OH$	Cl	0.018	0.007	0.028	7/6
20	(*R*)-$NHOCH_2CHOHCH_2OH$	Cl	0.032	0.023	0.046	52/23
21	(*S*)-$NHOCH_2CHOHCH_2OH$	Cl	0.013	0.018	0.029	38/30
22	(*R*)-$NHOCH_2CHOHCH_2OH$	F	0.029	0.045	0.072	107/28
23	(*S*)-$NHOCH_2CHOHCH_2OH$	F	0.019	0.058	0.048	115/49
24	3-OH-azetidine	H	0.030	0.032	0.097	15/13
25	3-NH_2-azetidine	H	0.007	0.037	0.039	55/64
26	(*R*)-3-OH-pyrrolidine	H	0.013	0.084	0.127	14/9

Table 3
Selected rat PK parameters for compounds **13**, **15**, and **20**[15]

Compd	CL (mL/min/kg)	Vdss (mL/kg)	MRT_{iv} (h)	MRT_{po} (h)	*F* (%)
13	52	2896	0.93	6.4	46
15	48	2102	0.72	7.4	19
20	7.1	361	0.85	4.1	40

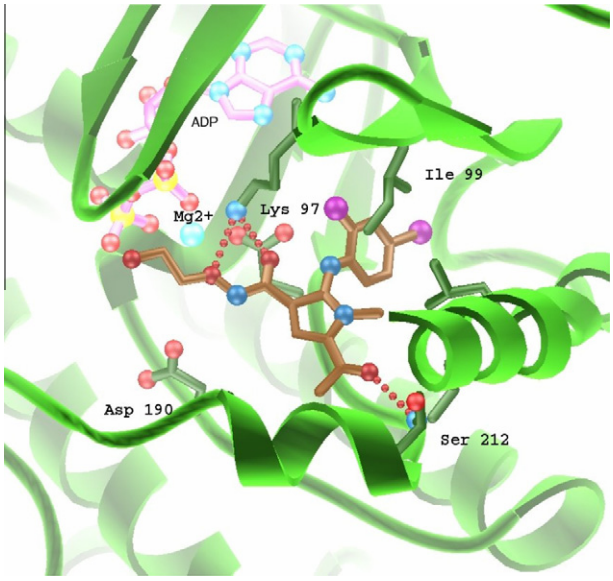


Figure 1. X-ray co-crystal structure of compound **13** in the MEK1 allosteric site.

high plasma clearance and reasonable oral bioavailability (46%). The *R*-isomer diol **15** showed lower oral bioavailability; whereas the 4-chloropyrrole **20** exhibited much reduced plasma clearance and improved oral bioavailability.

The co-crystal structure of compound **13** in the MEK1 allosteric site is shown in Figure 1.[16] The acetyl group oxygen makes important hydrogen bonding interactions with the backbone NHs of Val211 and Ser212 (2.9 and 3.0 Å, respectively). The acetyl methyl group packs tightly with the surface of the protein. As with the anthranilic hydroxamate inhibitors, the hydroxamide oxygens interact with Lys97. There is little density for the terminal hydroxyl group, indicating some flexibility in this region. The 2-fluoro-4-iodoaniline piece sits in the hydrophobic pocket formed by Ile141, Met143, Val127, and Phe209.

In conclusion, we have designed and characterized a series of novel pyrrole-based MEK inhibitors. Future reports will describe modifications of this scaffold for optimization of in vivo properties.

Acknowledgements

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9. MEK1 Enzyme Assay: Inhibition of compounds relative to MEK1 were determined using a cascade assay method in 384 well format under the following reaction conditions: test compounds serial diluted in DMSO were diluted into assay buffer (50 mM HEPES pH 7.3, 10 mM NaCl, 10 mM $MgCl_2$, 0.01% Brij35, 1 mM DTT) and added into ERK1, fluorescent labeled ERK1 substrate: IPTTPITTYFFFK-5FAM-COOH, and the reaction was initiated with 1 nM MEK1 and 400 μM ATP or 10 μM ATP. Reaction product was determined quantitatively by fluorescent polarization using progressive IMAP beads from molecular devices. Inhibition constants (IC_{50}) were calculated using standard mathematical models. An ERK1 assay was also conducted to rule out that inhibition was due to ERK1 in the cascade assay. Since all compounds tested showed almost identical IC_{50} when assayed at 400 μM or 10 μM ATP, only IC_{50} results assayed at 400 μM ATP were listed. Based on K_{mATP} for MEK1 at 20 μM determined using direct assay method (not shown), no potency shift when compounds were assayed at $10 \times K_m$ and $0.5 \times K_m$ ATP concentration indicated compounds were not ATP competitive inhibitors.
10. A375 and Colo205 EC_{50}s were generated using a cellular colorimetric MTS assay which measures newly produced NADH. Briefly, human cancer cell lines were seeded between 3000 and 10,000 cells per 96 well and incubated for 16 h in a humidified 5% CO_2 atmosphere incubator at 37 °C. Cells were then incubated with an eleven point dilution of test compound in duplicate for 72 h and subsequently assayed for NADH levels via the CellTiter 96-AQueous® kit (Promega) which utilizes a MTS tetrazolium salt conversion. The resulting colorimetric reaction was read on a spectrophotometer (Molecular Devices) at OD 490 nm and EC_{50} values of compound concentration vs. total NADH levels were calculated in Activity Base (IDBS). It is important to note the A375 and Colo205 cell lines both posses the BRAF(V600E) mutation making them reliant upon MEK signaling for survival. All compounds listed were also tested against the PC3 cell line whose survival is independent of MEK signaling and served as a control for MEK inhibitory selectivity. The EC_{50}s generated for all compounds listed were at a minimum 50-fold higher in the PC3 cell line.
11. Full experimental procedures for compounds **13**–**26** are contained within the following patent application: Adams, M. E.; Dong, Q.; Kaldor, S. W.; Kanouni, T.; Wallace, M. B. PCT Int. Patent Appl. WO 08/055236, 2008.
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14. Kinase Panel: Abl1, AKT3, c-RAF, CamK1Δ, CDK2/cyclinA, cMet, cSRC, EGFR, GSK3β, IR, JAK3, P38α, PDGFRβ, PDK1, PKCα, PLK3, Syk, Tie2.
15. Compounds were administered intravenously and orally at 1 and 5 mg/kg, respectively.
16. Protein Data Bank code is 3MBL.